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Responses to Charge questions on the draft Toxicological Review of RDX

1. **Literature search/study selection and evaluation.**
   The literature review is comprehensive and exclusion criteria are appropriate.

2. **Toxicokinetic modeling.** In Appendix C, Section C.1.5, the draft assessment presents a summary, evaluation, and further development of published PBPK models for RDX in rats, mice, and humans (Sweeney et al., 2012a; Sweeney et al., 2012b).

2a. Are the conclusions reached based on EPA's evaluation of the models scientifically supported? Do the revised PBPK models adequately represent RDX toxicokinetics? Are the model assumptions and parameters clearly presented and scientifically supported? Are the uncertainties in the model appropriately considered and discussed?

The changes to the PBPK model of Krishnan /Sweeney represent distinct improvements. Human metabolic rate constants were fitted from human data. Additionally, it is stated that in vitro data from rats and human metabolic studies were used, with scale-up to liver size based on microsomal protein. Oral absorption parameters were re-evaluated to take into consider different dosage forms. The further EPA evaluation also performed validation of the PBPK model using independent rat data sets, and provided goodness of fit parameters.

Specific comments:

1. Despite these improvements in the model, the data in rats is not fitted well and shows substantial deviations especially at early time points. This may reflect significant deviations of the data from the model absorption parameters, and possibly in clearance. Further optimization may improve fitting. For absorption, this might include permeability data using human intestinal CaCo2 cells or investigating other intestinal models. For elimination, hepatic intrinsic clearance is preferred over a rate constant. From *in vitro* microsomal and S9 studies reported by Cao, data is provided that can be used to calculate metabolic intrinsic clearance. Cao demonstrated that the intrinsic metabolic clearance in a microsomal preparation was greater in humans than in rats and mice. However, concentration-dependent studies were not performed, so this publication does not provide support for the assumption of linear clearance.
2. Clearance terms instead of first order rate constants (dependent on elimination and the apparent volume of distribution) would be more informative in the model. In vitro (Km/Vmax or intrinsic metabolic clearance) or derivation of intrinsic clearance from fitted clearance obtained from in vivo data may be used.
3. Clearance is assumed to be entirely by metabolism, which is a reasonable based on the accumulated data from many studies. Variability based on metabolic clearance could be built into the model (or by known variability in enzymes, such as CYP2B4 (a rabbit CYP which is suggested as a CYP involved in RDX metabolism). This corresponds to CYP2B6 in humans.
4. The role is metabolites in toxicity is discussed in the document, and due to a lack of data this is not included in the model. This is appropriate in the model due to the lack of data

and the fact that metabolites are not present to any extent in blood; however, there is no information on metabolites in brain and a potential role in neurotoxicity.

5. Tissue partitioning is mainly determined by in silico methods; more in vivo data would provide the justification for these values and decrease variability. The most important data would be partitioning into the brain since the major toxicity is neurological. Brain extracellular fluid concentration-effect relationships would be most informative and differences in binding to $GABA_A$ between rodents and humans could also be incorporated; this would drive the neurological effects. That being said, plasma/blood concentrations may be linearly related to brain concentrations, and may be used to drive toxicity, as proposed, based on limited correlations observed with brain and plasma data from animal studies and data from the child after poisoning (CSF data).
6. Protein binding of RDX is not mentioned in the document: it is the free concentration that would diffuse across the BBB in the absence of any active uptake processes and will be metabolized by the liver. This could lead to differences in brain/blood ratios in humans, and may be helpful in allometric scale-up of clearance.
7. Use of Simcyp or Gastroplus which both have PBPK modules could facilitate modeling.

2b. The average concentration of RDX in arterial blood (expressed as area under the curve) was selected over peak concentration as the dose metric for interspecies extrapolation for oral points departure (PODs) derived from rat data. Is the choice of dose metric for each hazard sufficiently explained and appropriate? The mouse PBPK model was not used to derive PODs for noncancer or cancer endpoints because of uncertainties in the model and because of uncertainties associated with selection of a dose metric for cancer endpoints. Is this decision scientifically supported?

Since we are focusing on chronic toxicity, AUC is more appropriate. Additionally, peak concentrations are not predicted well from the PBPK models.

Only the dose metric for seizures/convulsions provides reasonable certainty. That for cancer endpoints does not.

2c. In Section 2.1.3 of the draft assessment, an uncertainty factor of 10 for human variation if applied in the derivation of the RfD. Does the toxicokinetic modeling support the use of different factor instead?

There are a number of uncertainties, including clearance, brain concentrations, protein binding, susceptibility to seizures, affinity for binding to GABAA receptors, oral absorption parameters, and metabolite contribution to toxicity which limit certainty. Overall, until we have a better understanding of the variability of PK, PD, and relevant concentration-effect relationships, the uncertainty factor needs to be large.

3. **Hazard identification and dose–response assessment.**

3a. Nervous system effects

- **Nervous system hazard** (Sections 1.2.1, 1.3.1)**.** The draft assessment concludes that nervous system toxicity is a human hazard of RDX exposure. Please comment on whether the available human, animal, and mechanistic studies support this conclusion. Are all hazards to the nervous system adequately assessed? Is there an appropriate endpoint to address the spectrum of effects?

  There doesn't appear to be an appropriate endpoint to address the spectrum of neurological effects, at this time. EEG and memory evaluations may provide earlier clinical endpoints.

- **Nervous system-specific toxicity values** (Section 2.1.1). Please comment on whether the selection of studies reporting nervous system effects is scientifically supported and clearly described. Considering the difference in toxicokinetics between gavage and dietary administration (described in Appendix C, Section C. and in the context of specific hazards in the toxicological review), is it appropriate to consider the Crouse et al. (2006) study, which used gavage administration? Is the characterization of convulsions as a severe endpoint, and the potential relationship to mortality, appropriately described?

  Gavage and dietary administration will result in differences in Cmax and possibly in AUC (extent of absorption). Results from gavage studies are more reliable with respect to dose administration, since there is more variability in dietary consumption based on amounts of diet consumed, the influence of taste/smell of RDX on dietary intake, and effects of food on absorption. Particle size may influence bioavailability if aqueous solubility/dissolution is rate-limiting for absorption. Generally, gavage studies should be appropriate, except at very high doses where the initial blood concentrations of RDX may be very high resulting in toxicity. This may be due to a rapid rate of absorption and possibly saturation of first pass metabolism.

- **Points of departure for nervous system endpoints** (Section 2.1.2). Is the selection of convulsions as the endpoint to represent this hazard scientifically supported an clearly described? Are the calculations of PODs for these studies scientifically supported and clearly described**?** Is the calculation of the HEDs for these studies scientifically supported and clearly described?

  The calculations of HEDs are scientifically supported and clearly described.

3b. Kidney and other urogenital system effects

Points of departure for kidney and other urogenital system endpoints (Section 2.1.2). Is the calculation of a POD for this study scientifically supported and clearly described**?** Is the calculation of the HED for this study scientifically supported and clearly described?

The scientific data is variable with respect to toxicity involving the kidney and urogenital effects. However, the HED for neurological effects appears appropriate for kidney/urogenital effects based on scientific literature.

3c. Developmental and reproductive system effects

Points of departure for reproductive system endpoints (Section 2.1.2). Is the calculation of a POD for this study scientifically supported and clearly described? Is the calculation of the HED for this study scientifically supported and clearly described?

There is little evidence for reproductive toxicity.

3e. **Cancer**

(i) **Cancer hazard** (Sections 1.2.5, 1.3.2). There are plausible scientific arguments for more than one hazard descriptor as discussed in Section 1.3.2. The draft assessment concludes that there is *suggestive evidence of carcinogenic potential* for RDX, and that this descriptor applies to all routes of human exposure. Please comment on whether the available human, animal, and mechanistic studies support these conclusions.

I agree that the evidence is only suggestive.

(ii) **Cancer-specific toxicity values**

(iii) **Points of departure for cancer endpoints**

4. **Dose-response analysis.** In Chapter 2, the draft assessment uses the available human, animal, and mechanistic studies to derive candidate toxicity values for each hazard that is credibly associated with RDX exposure in Chapter 1, identify an organ/system-specific RfD, then selects an overall toxicity value for each route of exposure. The draft assessment uses EPA's guidance documents (see http://www.epa.gov/iris/basic-information-about- integrated-risk-information-system#guidance) in the following analyses.

4b. **Inhalation reference concentration for effects other than cancer** (Section 2.2).

The data does not support an inhalation reference concentration.

4d. **Inhalation unit risk for cancer** (Section 2.4). The draft assessment does not derive an inhalation unit risk because inhalation carcinogenicity data were not available, nor were toxicokinetic studies of inhalation of RDX available to support development of a inhalation PBPK model. If you believe that the available data might support an inhalation unit risk, please describe how one might be derived.

The available data does not support an inhalation unit risk.